Alternative Binder from microalgae : Algoroute project

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Algoroute Project

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Renewable energy sources are developed worldwide because of high oil prices and to limit greenhouse gas emissions. In this context, some groups have focused their work on vegetable oils, and particularly, on microalgae. The last decade has seen an increased scientific interest in the extraction of lipids from microalgae for the production of biodiesel. Microalgae present many advantages compared to other energy crops including a high growth rate, a high biomass production, and noncompetition with human food production. For economical and ecological reasons, the byproducts resulting from the microalgae culture have to be valorized. The Algoroute project is based on the valorization of byproducts resulting from the microalgae production. Could they be used as a binder for aggregates? In that context, the nature and composition of those byproducts need to be identified and rheological properties of different nature of compounds need to be measured. Interesting preliminary results have been obtained. Several fractions from microalgae have been extracted and characterized by infrared (IR), nuclear magnetic resonance (NMR) (^{1}H and ^{13}C), and gas chromatography–mass spectrometry (GC-MS). The complex modulus was also determined on these different fractions. The comparison of rheological and chemical analyses allows highlighting some chemical species that show thermo-dependent behavior comparable to asphalt.

INTRODUCTION

At the moment, many alternative binders, derived from biomass, are already developed by some firms like Colas or Shell. They are principally based on vegetable oils (e.g., linseed, rape). To enhance their viscosity, a natural resin can be added (*1*) or a chemical modification is necessary. Nevertheless, it would be advantageous to find a new alternative binder, based on nonfood crops.

The last decade has seen an increased scientific interest in the extraction of lipids from microalgae for the production of biodiesel. Microalgae present many advantages compared to other energy crops including a high growth rate, a high photosynthetic yield, and high lipid

content. Moreover, they do not compete with human feeding, as they can be grown on nonarable lands.

To reach the concept of "algo-refinery" all fractions of microalgae have to be valorized, and not only the triacylgycerols [which can easily be converted in biodiesel by transesterification (*2*)].

As asphalt is a co-product of the petroleum industry, it would be interesting to use byproducts of microalgae industry to produce an alternative road binder without harming the noblest uses of biomass.

Here, the authors propose to use some residues of a microalgae from which some proteins have been removed and to study the lipid fractions. To improve the properties of this potential binder, it is interesting to figure out at first its chemical composition. Then, relationships between composition from one hand, and rheological and mechanical properties from another hand, have to be highlighted. This approach is original and, to the authors' knowledge, no microalgae oil composition–rheological behavior relationship has been reported.

Algoroute project is included in the IFSTTAR Post Oil Pavement research program, dedicated to alternative binders for pavement. It is cofunded by Pays de la Loire region (France) and is a partnership between academic laboratories (such as CEISAM and GEPEA) and companies (such as Algosource Technologies and Alpha Biotech).

MATERIAL AND METHODS

Microalgae Preparation

Microalgae were obtained from Alpha Biotech in Assérac, France. The major part of water-soluble proteins was removed by centrifugation. Residue of microalgae, containing about 80% water, was frozen and then freeze dried for 1 week at –90°C.

Extraction and Fractioning Methods

A Soxhlet apparatus was used to extract lipids from microalgae. About 4 g of microalgae powder was loaded in a 70-mL cellulose thimber; 90 mL of n-hexane, chloroform, and chloroform–methanol (2:1, v/v) were successively used for 24 h. Solvents were removed under vacuum at 40°C. Oil yields were determined by gravimetry.

A sonication of the sample in CCl_4 allowed us to fractionate the oil in two parts: a CCl_4-soluble fraction and a CCl_4-insoluble one that were separated by simple filtration.

Fatty Acid Methylation

Fatty acid methyl esters (FAMEs) were prepared according to Morrison and Smith (*3*); 12 mL of boron trifluoride in methanol (14%) were added to 200 mg of the lipid extract. The mixture was heated in a sealed tube at 90°C overnight. FAMEs were extracted by hexane, washed with water, and dried over $MgSO_4$. The solvent was removed under vacuum at 40°C and FAMEs were dissolved in isopropanol for their analysis by GS.

Analytical Methods

Nuclear Magnetic Resonance

Samples were dissolved in a deuterated solvent (e.g., $CDCl_3$, MeOD, or D_2O). ^1H NMR spectra were recorded on a Bruker AVANCE (DPX 300 or 400) Ultrashield and ^{13}C NMR spectra were recorded at 75 MHz or 100.6 MHz. ^{31}P spectra were recorded at 121 MHz or at 161.97 MHz.

Gas Chromatography

GC was performed on a Agilent 6890 equipped with a flame ionization detector and a BPX70 capillary column (70% cyanopropyl dimethylpolysiloxane, 30 m x 0.32 mm ID, 0.25 µm film thickness). Helium was used as carrier gas at 1.3 mL/min. The oven temperature was 120°C, held for 4 min, then raised to 220°C at a 6°C/min rate and held at 220°C for 5 min. It was then raised to 250°C at a 15°C/min rate, and held at 250°C for 18 min. The injector and detector temperature were set at 250°C. The identification of fatty acids was performed by comparison with the retention time of standards.

Infrared

IR spectroscopy was carried out with an IR-TF Vector 22. Oil samples were dissolved in CCl_4 and placed into a KBr window for the acquisition. A KBr pellet was used for the analysis of solid samples (1% in weight).

Rheology

The complex modulus and phase angle of the samples were measured with a Metravib viscoanalyser DMA+450, in the shear mode, at a 1- to 125-Hz frequency range and 10°C-to-80°C temperature range.

RESULTS AND DISCUSSIONS

Soxhlet Extracts Composition

Three successive extractions were performed in order to fractionate lipids according to their polarity and chemical structure. For hexane, chloroform, and chloroform–methanol the extraction yields are 5%, 5%, and 12%, respectively, which lead to a 22% cumulative yield. Soxhlet extracts appears to be a highly viscous thermo fusible black paste (Figure 1).

Further sonication in CCl_4 showed the existence of an insoluble fraction (0% in hexane extract, 15% in chloroform extract, and 35% in chloroform–methanol extract).

The soluble fractions are only composed by free fatty acids (Figure 2, Table 1). Even though there is no physiological reason that microalgae could produce free fatty acids, we observed only the lipids by ^1H and ^{13}C NMR. This could be the result of an enzymatic lysis of complex lipids during the preliminary protein extraction process.

FIGURE 1 Soxhlet extract from microalgae.

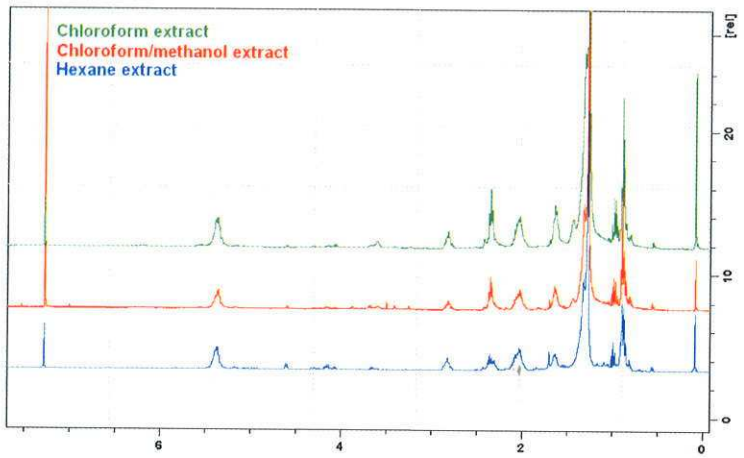

FIGURE 2 ^{1}H NMR spectrum comparison of the three extracts.

TABLE 1 ^{1}H NMR Assignment

δ (ppm)	Chemical Structure
0.9	$C\underline{H}_3 - (CH_2)_n -$ of ω6 and ω9
1	$C\underline{H}_3 - (CH_2)_n$ of ω3
1.2	$-(CH_2)_n-$
1.6	$-C\underline{H}_2 - CH_2 - COO -$
2.0	$-\underline{H}_2C-HC=CH-C\underline{H}_2-$
2.3	$-H_2C - COO -$
2.8	$= HC - C\underline{H}_2 - HC=$
5.3	$-HC=CH-$

To allow their analysis by GS, free fatty acids were analyzed as their methyl esters. At all, 18 fatty acids were identified but the main ones were palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid as seen in Figure 3 [classical ones found in microalgae (*4*)]. The only difference between the three extracts is the ratio between all those free fatty acids.

IR spectrum of the CCl_4 insoluble extract (Figure 4) shows some specific absorption bands. The broad band around 3,400 cm^{-1} is attributed to the O – H elongation of some hydroxyl groups. The two signals at 2,915 and 2,850 cm^{-1} are because of the C–H elongations of CH_2 and CH_3. The broad band center around 1,640 cm^{-1} can be attributed to the C = O elongation, similar to polyester. From comparison with literature data (*4*), we suspect the CCl_4 insoluble extract to be algaenans, a biopolymer highly resistant and nonhydrolysable, already encountered in microalgae (*5*, *6*).

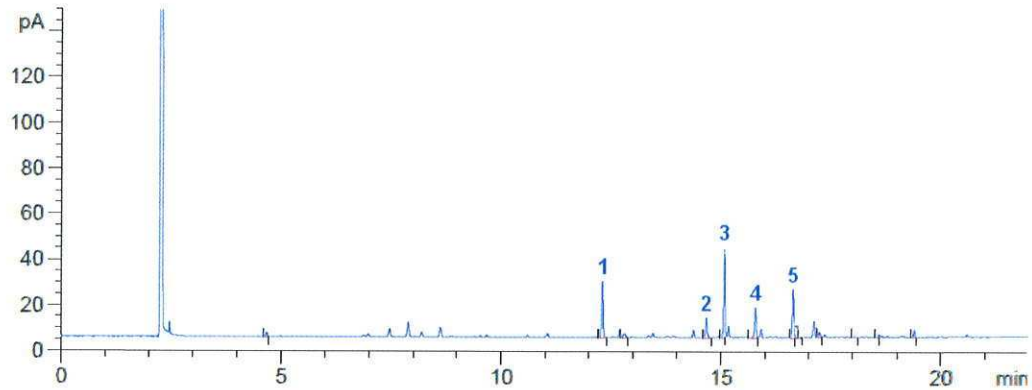

FIGURE 3 Typical GC profile of FAMEs obtained by Soxhlet extraction (1 = C16:0; 2 = C18:0; 3 = C18:1; 4 = C18:2; and 5 = C18:3).

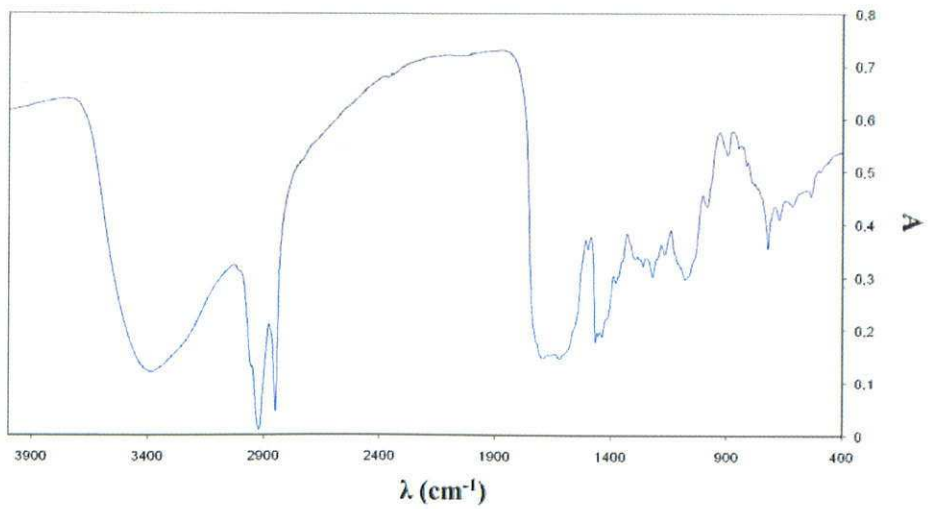

FIGURE 4 IR spectrum (KBr pellet) of CCl_4 insoluble extract.

Soxhlet Extracts Properties

In order to have a better understanding of the influence of each constituent (i.e., free fatty acids and algaenans) on the physical properties of the mixtures, the authors performed a rheological study (measurement of phase angle and norm of the complex modulus), varying the percentage of algaenans.

Three samples, with 0%, 20%, or 35% algaenans, were studied. An easy way to visualize the differences between all the samples is to depict the black curves (plotting phase angle φ against the complex modulus |E*|).

As seen on the Figure 5, the sample composed by only free fatty acids shows a discontinuity between all the isotherms. This is revealing a molecular reorganization at each temperature (i.e., melting of free fatty acids). An increasing from 0% to 20% of the algaenans content engenders a better arrangement of the isotherms, even if they are still discontinuous. A totally different behavior is observed at 35% of algaenans. In this case, as for asphalt, there is continuity between each isotherm, showing the thermo-rheological stability of the material.

The results suggest that it is possible to adjust the rheological properties of the material by adjusting the ratio algaenans versus free fatty acids. It could be an easy way to obtain alternative road binders with similar properties as asphalts. Indeed, complex modulus norm (measured at 1 Hz), was compared to two paving-grade asphalts. On the temperature range tested, microalgae show a thermosusceptibility similar to the one of asphalts (Figure 6). Consequently, it seems possible to obtain alternative road binders with the same rheological properties as asphalts.

Finally, a mix was manufactured with this microalgae oil (Figure 7). Even if more tests need to be done, it is particularly interesting from a qualitative point of view as the oil allowed to give a great consistency to the mix.

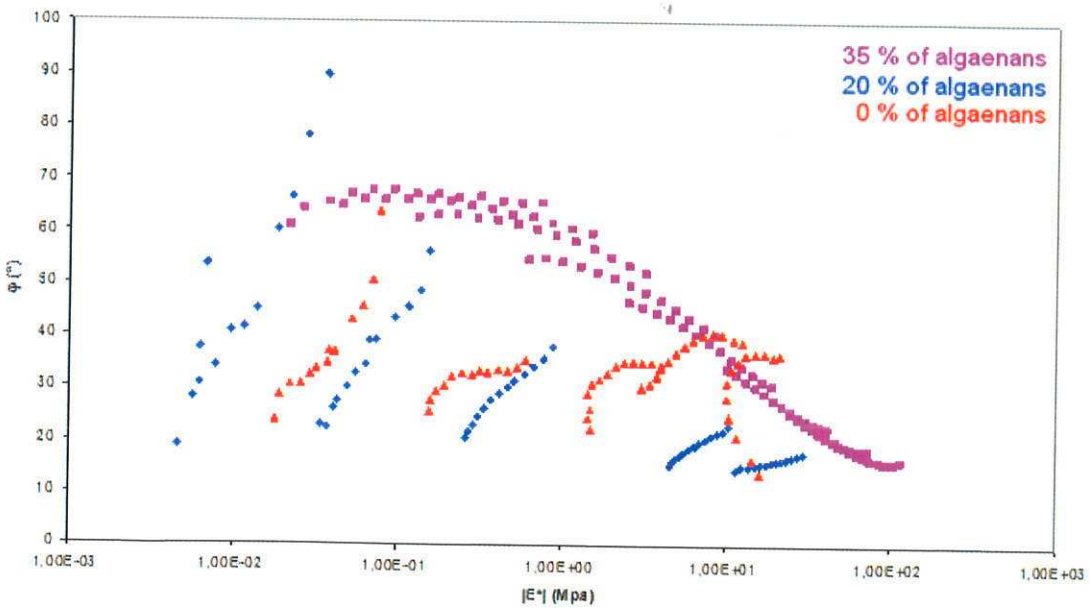

FIGURE 5 Evolution of black curves according to the algaenans content.

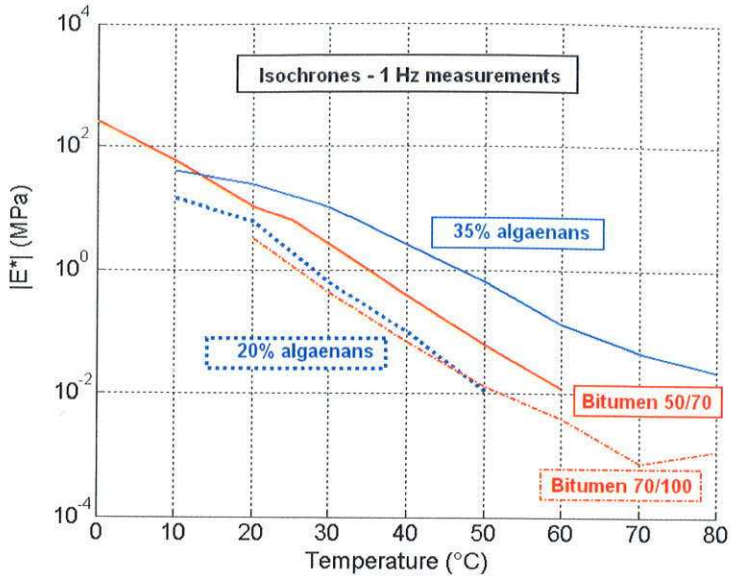

FIGURE 6 Rheological comparison between microalgae extracts and asphalt.

FIGURE 7 Mix manufactured with microalgae oil, using oedometric compaction system.

CONCLUSIONS

Through this study, the authors suggest that the design of microalgae-based road binders should be feasible. The isolated lipid fraction of the studied microalgae is made of a fatty acid polymer (commonly named algaenans) suspended in a free fatty acids oil. This thermofusible viscoelastic material shows rheological properties similar to those of asphalt. Moreover, a tuning of those properties can be achieved by adjusting the percentage of algaenans in the oil.

While only a fraction of the microalgae residue was addressed in the this paper, investigation of innovative and environmentally clean processes (i.e., low energy-demanding and solvent-free protocols) that would allow full valorization of the total residue is needed.

Other residues, from other strains of microalgae, will also be studied, in order to generalize those results.

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